# Advance of entomopathogenic nematodes

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**Abstract:** This paper summarized the history and present condition of studying and utilizing entomopathogenic nematodes at home and abroad, expounded its taxonomy, life cycle and the mechanism with symbiotic bacteria killing host insect. Taxonomy, mycelial form, pathogenic function and anti-bacteria function of symbiotic bacteria were introduced. Production and utilization of entomopathogenic nematodes, the characteristic genetic improvement by use of biological engineering technology, as well as the existing problem and applying foreground were also discussed.

**Keywords:** Entomopathogenic nematodes; Symbiotic bacteria; Genetic improvement; Biological control
**CLC number:** S763.1 **Document code:** A **Article ID:** 1007-662X(2001)04-0257-06

## Introduction

Entomopathogenic nematodes is a kind of insect's natural enemy of obligate parasitism, with wide host range, and can find host on its own initiative. Particularly it has good result for control of some soil pest and stem borers difficult to be controlled by chemical methods and also has characteristics of safety, non-poison, and lower cost. With pesticide pollution getting severe and drug-resistance of pest increasing, entomopathogenic nematodes as a biological resource of natural enemy with great potentials has drawn broad attention in the domain of international biological control (Cong 1999; Kaya *et al.* 1993).

Entomopathogenic nematodes has been successfully used in controlling some farm and forestry pest, such as *Otiorhynchus sulcatus*, *Pachraetus Litus*, *Odoiporus longicollis*, *Arbela dea*, *Carposina niponensis*, *Alissonolurn impressicalla*, *Holotrichia parallela*, *Blitoportha pallidipennis Reitter*, *Paranthrene tabaniformis*, *Phylloreta striolut*, etc. (Cong 1999).

In recently years, with fast development of science and technology and improvement of research methods, the studies on entomopathogenic nematodes has gained grand achievements and presented a wider application foreground. This paper mainly discusses the taxonomy, biological character, pathogeny, culture methods, symbiotic bacteria, and genetic improvement of entomopathogenic nematodes, meantime discusses its developments.

**Foundation item:** This paper was supported by Natural Science Foundation of Heilongjiang Province (No.C-9735).
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**Received date:** 2001-09-25
**Responsible editor:** Song Funan

## Taxonomy

Since a long period of time, the taxonomy of entomopathogenic nematodes has thrown into confusion, the traditional taxonomy mainly based on its morphological characters including body length, the distance from head to nerve ring, the distance from head to excretory pore, male copulatory spicule, and gubernaculums, etc.. However, the different strains and individual of the same specie also come into being considerable difference due to the variations of host, nourishment, culture temperature, and the time of gaining infective juvenile (Akhurst 1987). Up to 1978, Akhurst proposed that taxonomy of entomopathogenic nematodes by hybridization might provide reliable basis. Meantime by combining with taxonomical methods of morphological characters, biochemistry, and molecular biology, the taxonomy of entomopathogenic nematodes has a new breakthrough, which modified primary classification system. In two international symposiums of entomopathogenic nematodes holding in 1989 and 1995, the confusions in taxonomy of entomopathogenic nematodes were further clarified. According to Poinar (1979, 1983) classification system, up to 1998, *Steinernematidae* was divided into two genera, *Steinernema* and *Neoaplectana*, totally including 21 species. *Heterorhabditidae* had one genus (*Heterorhabditis*) and 9 species (Table 1) (Xu 1998).

As some new species were increasingly found, Akhurst (1995) thought that there still existed some problems in

taxonomy of entomopathogenic nematodes. For example, at present the new species were almost described based on morphological characters and less or no attention was paid to the symbiotic bacteria of nematodes, and also there often exist differences between the morphological analysis and analysis of molecular biology. The analysis of molecular biology was used mostly to phylogenetic tree and less to species identification. Therefore we need to do a quantity of work on the phylogenesis of entomopathogenic nematodes.

**Table 1. The species of *Steinernematidae* and *Heterorhabditidae***

| *Steinernematidae* | | | *Heterorhabditidae* |
|---|---|---|---|
| *Steinernema* | | *Nevaplectana* | *Heterorhabditis* |
| *S. longicaudum* | *S. monticolum* | *N. longicurvicauda* | *H. argintinensis* |
| *S. glaseri* | *S. intermedia* | | *H. bacteriophora* |
| *S. affnis* | *S. oregonensis* | | *H. brevicaudi* |
| *S. ritteri* | *S. carpocapsae* | | *H. hawaiiensts* |
| *S. bicornutum* | *S. kushidai* | | *H. hepiualius* |
| *S. anomali* | *S. scapterisci* | | *H. indicus* |
| *S. neocurtillis* | *S. kraussei* | | *H. megidis* |
| *S. puertoricensis* | *S. cubana* | | *H. marelatus* |
| *S. rara* | *S. bibionis* | | *H. zealandica* |
| *S. feltiae* | *S. riobravis* | | |

## Biological characters

The development of entomopathogenic nematodes has three stages: egg, larva and adult. The larvae have four instars, the 1st-instar, 2nd-instar, 3rd-instar, and the 4th-instar. The 3rd-instar larva can live outside host body and it is solely instar larva having infective ability, namely the infective juvenile. The infective juvenile has sheath that doesn't get off in the 2nd-instar, which has functions on resisting harmful environments and increasing virulence of nematodes, so it is also named daner juvenile. As coming across right host-pest, the infective juvenile enters into body of insect through natural entrances (such as oral cavity, anus and value), wound, and synarthridial membrane, and then release symbiotic bacteria from midgut enteric cavity of nematodes, which quickly reproduce in hemocele of host pest and consequently cause host death. The entomopathogenic nematodes mainly develops in tissue and bacteria cell of host, and reproduces offspring by mating after maturation. When developing to infective juvenile, with symbiotic bacteria, it comes out from dead body of insect and looks for new host.

The entomopathogenic nematodes and symbiotic bacteria typically benefit each other. The nematodes transfers symbiotic bacteria among host and protects symbiotic bacteria from environmental influence, and then protects symbiotic bacteria against defence system of host. The symbiotic bacteria supplies basic nourishment material for nematodes reproduction and produces toxin and ablastin, which provide well living conditions for nematodes. Symbiotic bacteria can't exist outside environment alone. It can't cause diseases of host insect after being swallowed. The nematodes needs symbiotic bacteria to kill host insect (Milstead *et al.* 1979; Boemare *et al.* 1993). In two nematodes families, the *Heterorhabditidae* has stronger dependence on symbiotic bacteria. It can't be cultured in vitro and can't kill insect without symbiotic bacteria, while *Steinernematidae* may culture in vitro, but its reproductive ability descend and pathogenicity apparently decrease (Akhurst 1982). Entomopathogenic nematodes may digest and use symbiotic bacteria but the infective juvenile don't digest it, their mutually beneficial relationship is close and obligate symbiosis (Gaugler 1980).

## Mechanism of pathogenicity

### Infective process

In humid environment, by using water film to make vertical motion and horizontal motion, entomopathogenic nematodes can independently search right host and enter into host hemocele through some natural entrances (such as oral cavity, anus and value), wound, and synarthridial membrane etc.. Entomopathogenic nematodes could find host on its own initiative by chemical stimulation of host self or other materials. Graugler's (1980) studies showed that the odor of host dejecta or $CO_2$ of host breath might have allured function for the infective juvenile. The uric acid, xanthine, allantoin, ammonia, and arginine in insect egesta have allure function for nematodes (Schmidt *et al.* 1979). In addition some insecticide, acaricide and plant juice also have alluring function (Ishibashi 1987).

The nematodes may take different infective-pathways for different hosts. The difference of insect body wall texture and stigmatic opening texture is one of the reason of making difference of infective ability for nematodes. The settlement and searching ability of the infective juvenile have something to do with not only temperature, humidity, pH, and soil construction but also host behavior and natural defence system. After infecting host body, the nematodes

movement must lead to damage of host tissue to some extent, the change and destruction of cell construction, till death (Yang 1998).

### Nematodes and symbiotic bacteria damaging host haemolymph

*Damage to host defence mechanism*

The effective entomopathogenic nematodes must avoid or damage various defence mechanism of insect. After being infected, host haemolymph has various reactions for defence. The host cell and other blood cell resist symbiotic bacteria in initial stage, and usually reach the maximum value of resistance in 3-12 h. After 24 h, symbiotic bacteria damage this reaction of resistance and reproduce largely, thus damaging main organs of host (Gaugler 1994). The allied body of nematodes and symbiotic bacteria has different immunoreactions to various haemolymph of host as follows: (1) symbiotic bacteria could endure or damage humoral peridium of host (Dunphy *et al.* 1985; Dunphy and Webster 1991; Breholin *et al.* 1990); (2) nematodes produce inhibitive factor of inducing enzyme, which could protect symbiotic bacteria (Han *et al.* 1990); (3) before invested by host cell, nematodes releases symbiotic bacteria and makes host died of septicaemia (Welch and Bronskil 1992).

*Damage to host haemolymph*

The symbiotic bacteria can not cause host to die by oral cavity, since it acts on haemolymph of host, but there exists difference in the virulence of symbiotic bacteria because the different species of insect have different resistant reaction and ability to nematodes. Yamanaka's (1992) researches indicated that injecting 1-3 symbiotic bacteria can kill *Galleria mellonella* Linnaeus, while *X. japonica* has no virulence for *Spodoptera liture*. The blood cells of infected insect have obvious change, quickly decreasing in number. Symbiotic bacteria reproduce rapidly, and the number of bacteria exceeds ten millions per milliliter haemolymph as host on the brink of death, while the blood cells of host decreases by over 90 percent (Xiao 1990). As a result, a great quantity of nourishment of host is consumed and at last the host dies. This process mentioned above is a result of joint action of nematodes and symbiotic bacteria. In addition, the secondary metabolic product of symbiotic bacteria also directly influences normal function of host blood cells. The physiological and biochemical indices of host haemolymph have obvious pathological changes, such as the decrease of the contents of haemoproteins and blood sugar, and the strengthening of the active of esterase (Xiao 1990).

### Toxin and secondary substances produced by nematodes and symbiotic bacteria

The reason why entomopathogenic nematodes and symbiotic bacteria could kill host is that they can produce toxin. Burman (1982) found that *N. carpocapsae* could produce toxin periodically in the course of culture and reach the highest during combining period of the 4th-instar and adults. Besides directly acting on haemolymph of host, toxin might damage defence mechanism and immunity system of host, and make symbiotic bacteria quickly reproduce in haemolymph, and then make host die. Symbiotic bacteria can produce endotoxin and exotoxin in the course of growth (Kamionek 1993). The endotoxin is lipopolysaccharides of cell wall, which could stimulate degradation of host haemolymph (Dunphy and Webster 1989). The exotoxin is exocellular enzyme of cell (Clarke 1995). Its active might be related to exocellular enzyme of symbiotic bacteria, yet the toxicity for insect has not been verified (Boemare and Akhurst 1988).

The symbiotic bacteria of nematodes can produce many kinds of microbiostatic substances in the process of metabolism, which can prevent microorganism from enteron and soil from infecting dead body of insect and ensure the nourishment required for growth and reproduction of nematodes. Some secondary metabolic products have effect of poison on insect and result in anti-feeding of insect. McInerney and Gregson (1991) separated six disulfide pyrroline ramifications from metabolic products of T319 strain of *X. bovinii*, and carried on insecticidal test for group Ⅱ. These ramifications had 100% lethal rates for *Heliochis punctigera*. With low dose, the larvae weight obviously lightened, and feeding may be inhibited.

## Symbiotic bacteria

### Taxonomy and type

Bovien (1937) firstly reported that *S. bibionis* in infecting period had symbiotic relationship with bacteria. After then, symbiotic bacteria was continuously separated from new specie and new strains of nematodes. Poinar (1965) included them in *Achromobacter*, and later this genus was cancelled and the *Xenorhabdus* was set. Khan *et al.* (1977) firstly separated *X. luminescensjun* strains from *Heterorhabditidae*. These strains could produce sharp fluorescence, but the symbiotic bacteria from *Steinernematidae* could not do that. The symbiotic bacteria from these two nematodes families were carried at the front of enteron of the infective juvenile. The symbiotic bacteria with *Steinernematidae* was *X. nematophilus* and that with *Heterorhabditidae* nematodes was *X. luminescence*. Boemare (1993) included *X. luminescens* into *Photorhabdus*. Akhurst (1980) found bisexuality of symbiotic bacteria, namely primary type and secondary type. The primary type was separated from the body of infective juvenile and often changed into secondary type by virtue of instability outside body culture. Vacuum frost drying method was used to preserve bacteria of primary type. The distinction for primary type and secondary type was that there exists difference in colonial morphology, pigment excreting, absorption of bromophenol blue and methyl red, and glutinosity etc.,

but no difference in toxin. The bacteria of primary type could translate a serial of materials into culture medium adapting to growth and reproduction of nematodes, but couldn't the secondary type, so that for mass culturing, the culture medium must be inoculated with the symbiotic bacteria of primary type.

**Pathogeny and antibacterial action of symbiotic bacteria**

During discussing pathogenic action of symbiotic bacteria for insect, researchers formerly considered that the crystalline inclusion in bacteria strain that was similar with parasporal crystal of *Bacillus thuringiensis* Berliner (Grimont *et al.* 1984) had pathogenic action, but the virulence test didn't support this view (Buecher *et al.* 1989). Dunphy and Webster (1989) proved that the lipopolysaccharide excreting from pericellular membrane of *X. nematophilus* was the one of lethal factors of *Galleria mellonella* Linnaeus.

## Production and application

The research and application of entomopathogenic nematodes began in 1930's. In initial stages, its reproduction and culture mainly depended on culture in vivo, but not suitable for mass propagation due to high cost, which restricted the practical application of entomopathogenic nematodes all time. Until the 1980's, chick viscus as culture medium was inoculated with symbiotic bacteria to mass-reproduce entomopathogenic nematodes outside body and the liquid-culture technique was also employed for production of nematodes, which exposited wide foreground for research and application of entomopathogenic nematodes and made commercialization production flourishing again (Bedding 1981, 1984; Pace *et al.* 1984; Buecher and Popiel 1989). Now a large scale of artificial culture of nematodes mainly adopted solid culture and liquid culture, with characteristics of easy control of parameter, simple culture process, suitable for mass-production, and highly productive efficiency etc.. In recent years, domestic and foreign experts carried on detailed research and optimized combination for culture system. Mass-production of nematodes developed rapidly (Pace 1986). In the end of 1970's and the early 1980's, entomopathogenic nematodes were introduced, explored and researched in China, and its culture underwent the developing process from culture in vivo to culture in vitro and from bacteria-free to monomycelial culture (Yang 1995). Now Guangdong Research Institute of Insect and the Biological Control Research Institute of China Academy of Agriculture Science had a certain ability of large-scale production.

The development of large-scale manufactory culturing technology stimulates the applied research of entomopathogenic nematodes. At present, some companies of America, Australia, Holand, Canada, etc. can provide nematodes preparation, and more than one hundred of strains have realized commercialization production. China has had the successful example by use of nematodes to control some soil pest and stem borers difficult to control by chemical methods. Meantime researches have been carried out on the synergistic action of jointly applying the nematodes and many kinds of protective agents such as evaporating additive, water-holding additive, binder and ultraviolet protectant etc.. It was found that the protective additive had obvious effect on protecting nematodes and increasing control results (Liu *et al.* 1990), and then the synergistic action of jointly applying nematodes and chemicals was also studied, and it was found that the acephate, zineb, phoxim, deltamethrin, O-dimethoate etc. were safety for *Steinernematidae* and obviously increased controlling effects of field.

## Genetic improvement

With the continuous development of gene engineering technology and demands of control, advanced biological engineering technology has been applied to genetic improvement of pathogenic nematodes, which made nematodes and symbiotic bacteria give full play to biological control. In recent years, through gene engineering of selective breeding, the following studies were carried on.

(1) Increasing the nosogene of nematodes for host: Lindergren (1987) selected a new strain, which the $LT_{50}$ (median lethal time) of *Galleria mellonella* Linnaeus was 3 d ahead of normal, culturing cycles 6 d short of normal, production increased by 34 percent, and the virulence for *Lymantria dispar* L. increased by two times.

(2) Enhancing the ability of finding host: North America strain and New Zealand strain of *S. carpocapsae* were tested on finding host, the result indicated that the difference between strains was significant, but the ability of finding host didn't reach applied value (Gaugler *et al.* 1989).

(3) Adapting to temperature: Burman and Dye reported that under special temperature the infective juvenile of *S. carpocapsae* could still develop and infect insect normally and showed stronger adaptation.

(4) Tolerating ultraviolet: the test of the strains of North American, Europe and New Zealand showed that the $LD_{50}$ (median lethal dose) of most strains was 5.71-7.26 min for the ultraviolet endurance and genetic variability and didn't reach ideal value.

In the process of genetic improvement of character by gene engineering, Fire (1993) had succeeded in transferring the exogenous DNA into nematodes. Foder successfully induced the mutant of *S. carpocapsae*. This mutant can resist avermection. Frackman (1989) set up a DNA gene bank of the symbiotic bacteria *X. luminescens* of *Heterorhabditidae* with pUC18 plasmids for carrier. The luminous gene was cloned, which can resist assault of bacteriophage XLP, meantime the gene of *Xenorhabdus* was cloned and expressed in *E. coli* and luminous bacteria

of *Heterorhabditidae* (Xu 1995).

## Problems and expectation

With the development of ecological control theory, entomopathogenic nematodes could further meet the demands of biological control, with merits of rapidly propagating, easily hybridizing and gene improving, and have wide foreground of development. But some factor limited its application, such as temperature, humidity, ultraviolet, the specificity of symbiotic bacteria, the instability of primary type etc.. So we need to do a large quantity of basic and applied researches, including application and protection techniques, selecting and improving new strains by genetics, and enlarging the scope of disinsection, in order to sufficiently exploit the potentials of entomopathogenic nematodes in controlling pest.

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